A Kairomone Based Attract-and-Kill System Effective Against Alfalfa Looper (Lepidoptera: Noctuidae)

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ABSTRACT A chemical lure derived from flowers that are visited by moths attracts male and female alfalfa loopers, *Autographa californica* (Speyer) (Lepidoptera: Noctuidae). This feeding attractant is dispensed from polypropylene bottles that provide controlled release for several weeks. A killing station was tested in the laboratory, in a screenhouse, and in the field in combination with this lure as an "attract-and-kill" system. Starved alfalfa looper adults (moths) were strongly attracted to the attract-and-kill station in a flight tunnel, and 90.9% of female moths and 87.6% of male moths that contacted the station died. In commercial fields of alfalfa hay, female moths captured in monitoring traps were reduced by 80–93% in plots receiving 125 attract-and-kill stations per hectare. In screenhouse trials using two attract-and-kill stations per screenhouse, oviposition on potted lettuce plants by starved female alfalfa looper moths was reduced by 98.5%. Moths were less likely to be attracted to lures when provided sugar before flight tunnel assays, and oviposition by fed moths was much less affected by attract-and-kill stations in screenhouse trials, compared with starved moths. This method has potential as a means to manage alfalfa looper populations in vegetable and other agricultural crops. However, consideration must be given to competing food and odor sources in the field.

KEY WORDS attract and kill, attractant, feeding, moth, behavior

The alfalfa looper, *Autographa californica* (Speyer) (Lepidoptera: Noctuidae) is a widely distributed pest of numerous crops in western North America (Eichlin and Cunningham 1978). The larvae damage cole crops such as cabbage and broccoli, and they defoliate potatoes, peas, sugarbeets, alfalfa, beans, mint, spinach, and other crop plants (Brewer 1995, Robinson et al. 2002). Although alfalfa looper populations on crop plants may be held in check by natural enemies, they are often managed by foliar insecticide applications that target the early instars (Baird and Homan 1996). Potential disadvantages to these insecticide applications include concerns for their negative impact on human health, natural enemies, economic costs, the environment, and residues on food crops. A possible alternative to pesticide sprays is the use of baits or attract-and-kill technologies that combine an attractant or attractive material with a toxicant. Such an approach would permit reductions in the amounts of pesticides used and would minimize pesticide contact with the environment, the crop, and beneficial organisms. This approach has been used against the boll weevil, *Anthonomus grandis grandis* Boheman (Villavaso et al. 1988); codling moth, *Cydia pomonella* (L.) (Charmillot et al. 2000); and apple maggot, *Rhagoletis pomonella* (Walsh) (Prokopy et al. 2000, Bostanian and Racette 2001), among others.

Management of pest populations directly with chemical attractants, whether for trapping, baiting, or attract-and-kill approaches, often involves the use of lures that are effective for females if not for both sexes (Landolt 1997). Removal of males from a population, which is the usual result of trapping with moth sex pheromones, may not have a significant effect on reproduction unless a large percentage of the male population is killed, because generally males mate more frequently than females. Thus, a small percentage of the male population will serve to mate with most sexually receptive females. The attraction and killing of females, however, should directly impact the reproductive potential of a pest population, by reducing the number of eggs laid and consequently reducing the number of larvae infesting the crop in the subsequent generation. The development of an attract-and-kill technology might be enhanced with chemical lures that are effective in bringing females into a target. An example is the attract-and-kill system for the boll weevil, which uses the weevil's aggregation pheromone together with a host kairomone to attract males and females to a pesticide-coated target (Villavaso et al. 1988). A similar approach is used in an attract-and-kill method for the apple maggot fly, which uses a host attractant and a visual target to attract flies, and a pesticide formulation coating the target to kill at-

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tracted flies (Prokopy et al. 2000, Bostanian and Racette 2001).

Although the majority of chemical attractants for moths are female-produced sex attractants that lure males (Landolt 1997), female moths may use chemical odorants to locate and select mates (Willis and Birch 1982, Landolt and Heath 1989), host plants (Landolt 1989, Light et al. 2001), and food (Utrio and Erikson 1977, Haynes et al. 1991). Food for moths often consists of sweet materials such as sap, fruit juices, honeydew, and floral nectar (Norris 1936). Looper moths in the noctuid subfamily Plusiinae are particularly known for their visits at flowers, and for their attraction to the odors of those flowers (Grant 1971; Cantelo and Jacobson 1979; Haynes et al. 1991; Heath et al.1992a; Landolt et al. 2001, 2006; Plepys 2001; Landolt and Smithhisler 2003). The alfalfa looper visits flowers of *Berberis aquifolium* Prursch (Oregon grape) in early spring (Landolt and Smithhisler 2003), and it is attracted by two compounds emitted by the flowers: phenylacetaldehyde and β-myrcene (Landolt et al. 2006). This chemical blend attracts both male and female alfalfa loopers and provides the opportunity to develop population management strategies involving the killing of the females at traps or stations.

We report here the attraction and mortality of female alfalfa looper moths in response to attract-and-kill stations baited with a floral-based lure, reductions of moths in monitoring traps in fields with these same stations, and reductions in oviposition by moths in a screenhouse in the presence of attract-and-kill stations. We also assess the impact of prior access by moths to sugar on both attraction to the floral lure and the efficacy of the attract-and-kill station in preventing infestations on plants.

Materials and Methods

Attract-and-Kill Stations. Stations were modified from those of E. R. Mitchell (USDA–ARS Gainesville, FL; deceased) and Landolt (2002) (Fig. 1). They consisted of 1) a white cone-shaped target for the moth to contact; 2) an attractant dispenser at the base of the cone; and 3) a killing agent applied to the cone target, all integrated into a single unit. The cone is a white plastic badminton birdie (shuttlecock) measuring 7.6 cm in height with a 6.5-cm-wide opening. The red rubber bulb that forms the 2.5-cm-diameter shuttlecock base was replaced with an 8-ml polypropylene bottle (Nalgene 2006-9025, Fisher, Pittsburgh, PA) as the dispenser for the attractant. Each bottle was loaded with 5 g of the feeding attractant, a combination of 2.2 g of phenylacetaldehyde and 2.8 g of β-myrcene (Sigma-Aldrich Chemicals, Milwaukee, WI). The attractant mixture was added to cotton balls placed in the bottom of the bottles. A 3-mm-diameter hole was drilled into each bottle lid to provide a consistent controlled release of the volatilized chemicals from the bottle, with an estimated release rate of 43 μg/h at 20–22°C (Landolt et al. 2001, 2006). The bottle was then attached to the base of the shuttlecock with Hot Glue, with the lid and hole of the bottle facing into the cone. The internal and external surfaces of the cone were coated with Teflon Hitch Ball Lube grease (Reese Products Inc., Elkart, IN) mixed with 7% by weight technical grade Permethrin (FMC Corp., Princeton, NJ).

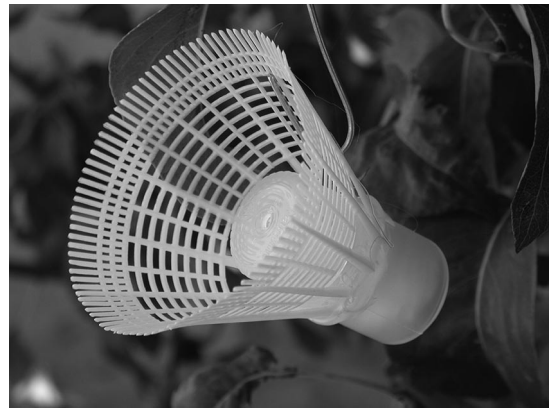

Fig. 1. Attract-and-kill station, made up of a badminton birdie or shuttlecock, with a polypropylene bottle dispenser as the lure in place of the rubber bulb of the shuttlecock.

Insect Rearing. Alfalfa looper colonies were started annually with eggs obtained from female moths captured in a light trap at the USDA–ARS Yakima Agricultural Research Laboratory, near Wapato, Yakima County, WA. These females were collected in mid-March to mid-April 2004 and 2005. Eggs were surface sterilized with a 0.15% bleach solution (1.5% Chlorox) and set up for hatching under room conditions (22 ± 1°C and 60% RH). Newly hatched larvae were transferred to 30-ml plastic cups (Solo Cup Company, Highland, IL) containing 10–15 g of artificial diet (Cabbage Looper Diet, Southland Products, Lake Village, AR). Larvae were held, one per cup, on this diet through to the pupal stage, with additional or replacement diet added as needed. Pupae were removed from diet cups, separated by gender, and placed into screened cages (20.0 by 20.0 by 20.0 cm), with males in one cage and females in a second cage. Cotton balls soaked with water or a sugar water solution were placed in 5.4-cm-diameter plastic petri dishes that were set on the floor of each cage, providing moth access to water and sugar water. Pupae were moved to new cages daily, providing discrete age cohorts of male and female moths in separate cages. Three male moths and one female moth, when 2–3 d old, were placed into a 450 ml clear plastic cup with a screened lid (water and sugar water provided) to obtain eggs. Eggs laid in those cups were surface sterilized with 0.15% bleach, and newly hatched larvae were set up on artificial diet as described above. Excess moths not needed for colony maintenance were used in experiments.

Flight Tunnel Evaluation. A flight tunnel experiment determined rates of attraction and mortality of moths in the presence of attract-and-kill stations and assessed differences in attraction responses and mortality of females versus males, and fed females versus starved females. The flight tunnel walls, floor, and ceiling were of Plexiglas. The tunnel was 0.8 m in width

by 0.8 m in height by 1.8 m in length. Air was pulled through the tunnel at 0.22 m/s. Moths were held in a controlled environment room on a 16:8 (L:D)-h reversed light cycle, with lights off at 1000 hours (P.S.T.) and lights on at 1800 hours to facilitate conducting experiments during normal working hours. Room temperature and humidity were 25 ± 1°C and 55 ± 3% RH. Tunnel effluent was vented outside the room, after passage through a charcoal-coated fiberglass filter to remove exhausted odorants. Moths were tested when 2 or 3 d old and were unmated. Males and females were tested on different days. Moths to be assayed were placed in the flight tunnel room in a screened cage one hour before the experiments were begun. All testing was conducted between 1100 and 1500 hours, 1–5 h into the scotophase of the light cycle. Individual moths were placed in a 20-ml polystyrene vial with an open end and a screened end. The vial was hung horizontally, by using a small wire, from a metal ring stand near the center of the downwind end of the wind tunnel, positioned with the open end of the vial upwind. The attract-and-kill station was suspended by wire from a ring stand at the center of the upwind end of the flight tunnel. Each moth was observed for 3 min after placement in the tunnel and was then captured and held for 24 h in a capped 20-ml polystyrene vial. For the 3-min assay, moths were scored for plume tracking and contact with the station. Plume tracking was evident as direct upwind flight or zigzagging upwind flight toward the station. At 24 h after the assay, moth survival and mortality were recorded.

Female moth behavior and mortality were evaluated for the following three treatments: unmated female moths held without sugar 24 h before testing (starved) and without an attract-and-kill station in the flight tunnel, unmated female moths without sugar 24 h before testing and with an attract-and-kill station in the flight tunnel, and unmated female moths provided sugar during the 24 h before testing (fed), and with an attract-and-kill station in the flight tunnel. This experiment was repeated using males. Six sets of five moths were tested for each treatment and sex, with each set of assays conducted on a different day. Moths were used in assays once. The tunnel was aired with the blower on for at least 12 h after each set of assays to minimize risk of contamination of the tunnel walls with the floral lure. Data were arcsine transformed, and after data assumptions were verified, treatment comparisons were made with an analysis of variance (ANOVA) followed by a least significant difference (LSD) test to determine differences between treatments for each sex.

Field Evaluations. Experiments tested the hypothesis that placement of attract-and-kill stations in the field would kill a significant proportion of the population of female alfalfa looper moths. These experiments were conducted in commercial fields of alfalfa, *Medicago sativa* L., in Yakima and Benton counties of Washington during the 2003 and 2004 growing seasons. These were fields of alfalfa grown for hay, and the alfalfa was cut and baled on a schedule through the season. Fields were monitored for alfalfa looper moths before experiments were begun. Square plots (2.0 ha) were established and monitored early in the season, by using one floral lure (phenylacetaldehyde with β-myrcene) trap per plot beginning in mid-May in both 2003 and 2004. Traps were multi-colored UniTraps (Universal moth trap) baited with a floral lure and containing a 6.5-cm^2 piece of Vaportape (Hercon, Emigsville, PA) stapled to the inside wall of the bucket. The floral lure in monitoring traps was the same as the floral lure used in the attract-and-kill stations. Floral lure bottles were suspended upright in the bucket of the trap, by a thin wire. Traps were hung on wire from stakes at a height of ≈0.5 m. Field experiments were begun when monitoring traps indicated that suitable numbers of moths were present. Attract-and-kill stations were placed in the field by attaching them to 1-m lengths of 8-gauge wire with one end of the wire driven into the soil and the other end bent into a loop from which the station was suspended. Stations were suspended ≈12 cm above the alfalfa crop canopy.

Monitoring of alfalfa looper moths during field trials was accomplished with two sex pheromone-baited traps per plot and two floral lure-baited traps per plot. Traps were Universal moth traps as described above. Sex pheromone lures were 1.1-mg loads of a 91:9 ratio of (Z)-7-dodecenyl acetate and (Z)-7-dodecenol, respectively, in red rubber septa (West Company, Lionville, PA). Pheromone lures were placed in the plastic baskets provided with the traps under the centers of the trap lids. Floral lures for traps were as described above. Traps were checked and emptied daily (2003) or every other day (2004). Trap contents were placed into Ziplock plastic bags (S.C. Johnson and Sons, Racine, WI) and transported to the laboratory where they were stored in a freezer. Insects were later sorted, identified, and sexed.

Each 2.0-ha plot was divided into four equal quadrants with a monitoring trap placed in the center of each quadrant. Pheromone traps were placed in the northwest and southeast quadrants with floral lure traps in the northeast and southwest quadrants. Each trap was 73 m from the nearest adjacent trap.

In 2003, a field experiment was conducted to assess the ability of the attract-and-kill stations to kill a significant proportion of the moth population. Tests were conducted for 14 d. During the first 7 d of the test, all plots were monitored with floral lure traps and sex pheromone traps, before the deployment of attract-and-kill stations. On the eighth day, 250 attract-and-kill stations were deployed per treated plot, and control plots did not receive stations. Plots were monitored daily for another 6 d after deployment of stations. Stations were distributed in a grid format, positioned uniformly throughout the 2-ha plots. Treated plots were paired with control plots in the same alfalfa fields. Four replicates of this experiment were conducted from late May through mid-August of the 2003 growing season. After data assumptions were verified, data were analyzed by a pretest/posttest repeated measures analysis (Brogan and Kutner 1980). Statistical analyses compared numbers of female and male

Table 1. Mean ± SE percentages of alfalfa looper moths attracted to and contacting an attract-and-kill station with a floral chemical lure in a flight tunnel

Treatment comparison	% plume tracking		% contacting source		% mortality	
	$\bar{x}$ ± SE	*P* value	$\bar{x}$ ± SE	*P* value	$\bar{x}$ ± SE	*P* value
Female moths						
Control	0.0 ± 0.0		0.0 ± 0.0			
Treatment, starved moths	83.3 ± 6.2	<0.01	73.3 ± 6.7		66.7 ± 21.1	<0.01
Control	0.0 ± 0.0				0.0 ± 0.0	
Treatment, fed moths	26.7 ± 4.2	<0.01	20.0 ± 5.2		16.7 ± 6.2	<0.01
Treatment, starved moths	83.3 ± 6.2		73.3 ± 6.7		66.7 ± 21.1	
Treatment, fed moths	26.7 ± 4.2	<0.01	20.0 ± 5.2	<0.01	16.7 ± 6.2	<0.01
Male moths						
Control	0.0 ± 0.0				3.3 ± 3.3	
Treatment, starved moths	60.0 ± 5.2	<0.01	53.3 ± 4.2		46.7 ± 4.22	<0.01
Control	0.0 ± 0.0				3.3 ± 3.3	
Treatment, fed moths	30.0 ± 4.5	<0.01	20.0 ± 7.3		16.7 ± 6.2	<0.01
Treatment, starved moths	60.0 ± 5.2		53.3 ± 4.2		46.7 ± 4.22	
Treatment, fed moths	30.0 ± 4.5	<0.01	20.0 ± 7.3	0.01	16.7 ± 6.2	<0.01

Control assays were with no attract-and-kill station in the flight tunnel. Treatment assays were with an attract-and-kill station in the flight tunnel.

alfalfa looper moths captured before and after deployment of killing stations.

In 2004, an experiment again assessed the efficacy of attract-and-kill stations in reducing numbers of alfalfa looper moths in fields of alfalfa. In addition, this experiment aimed to evaluate the effects of attract-and-kill stations on reproduction by sampling numbers of alfalfa looper larvae and to evaluate the effectiveness of killing stations over a longer period. Several fields were monitored with a floral lure trap beginning in mid-May 2004 to determine the presence of suitable numbers of moths for the start of experiments. Attract-and-kill stations were deployed in plots on day 1 of the experiment. Paired experiments were replicated four times from mid-May to mid-August 2004. Again, treated plots received 125 attract-and-kill stations per hectare (250 stations per plot). Immediately after station deployment, plots were monitored with the floral lure and pheromone traps for the 20-d duration of the experiment. Larvae were monitored with sweep net sampling 4, 8, 12, 16, and 20 d after the start of each experimental replicate. Sampling was done by dividing each plot in quadrants and executing 200 sweeps in random locations within each quadrant. A 54-cm-diameter sweep net (BioQuip Products, Palo Alto, CA) was used for sampling. Data were analyzed after assumptions were verified, by an ANOVA with repeated measures to compare numbers of moths captured in treated plots and untreated plots. Data for larvae sampled were not analyzed because of low numbers captured in the sweep net samples.

Screenhouse Evaluation. The hypothesis that oviposition by female alfalfa looper moths, and subsequently the numbers of larvae, are reduced by deployment of the attract-and-kill stations was addressed by releasing moths in a screenhouse treated with attract-and-kill stations. Alfalfa looper moths that had been reared in the laboratory as described previously were released into a screenhouse that was 7.4 m in length by 2.9 m in width by 4.9 m in height. One hundred moths (75 males, 25 females) were released per test replicate. All moths used in this experiment were 1–3 d old when released into the screenhouse and had been held in screened cages with water on cotton, in a greenhouse exposed to the natural light cycle. Lettuce plants in pots were used as host plants for oviposition. Lettuce was chosen because of its excellent suitability as a host for alfalfa looper larvae (P.J.L., unpublished data), and its rapid growth. Lettuce plants were ≈20 cm in height when used in screenhouse tests. One hundred lettuce plants in individual 15-cm-diameter pots were used in each assay. Pots were arranged on the floor of the screenhouse in two rows containing 50 plants each. Each row consisted of 10 black plastic trays 54 by 36 cm and 7 cm in height, each holding five potted plants. Experiments lasted 7 d after the release of moths into the screenhouse with the lettuce plants. At the end of that time period, eggs and larvae on plants were counted. Treatments were 1) no attract-and-kill stations in the screenhouse and moths not provided sugar before release, 2) two attract-and-kill stations in the screenhouse (equivalent to125 attract-and-kill stations per hectare) and moths provided sugar before release, and 3) two attract-and-kill stations in the screenhouse and moths not provided sugar before release. Each treatment was replicated three times during 2005. Numbers of eggs and larvae on lettuce plants were combined as numbers of offspring and the data analysis was performed using ANOVA, followed by an LSD test, after data assumptions were verified.

Results

Flight Tunnel Evaluations. Both starved and fed female alfalfa looper moths exhibited plume tracking in the presence of attract and kill stations (Table 1) ($n = 6$, df = 17, $F = 183.8$, $P < 0.01$ for starved moths; $n = 6$, df = 17, $F = 41.7$, $P < 0.01$ for fed moths). In addition, most starved female moths that were attracted to stations contacted those stations. When comparing data for starved and fed female moths,

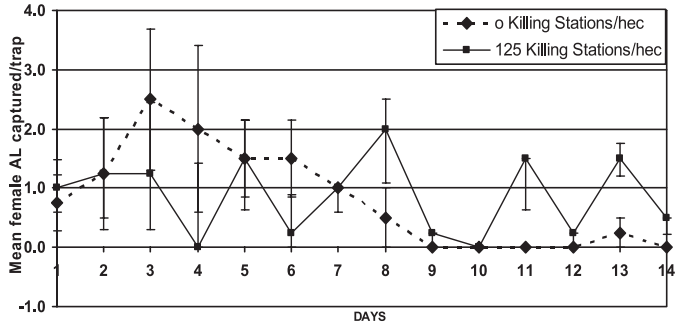

Fig. 2. Mean ± SE ($n = 4$) female alfalfa looper moths captured with feeding attractant-baited traps during the 2003 growing season. Attract-and-kill stations were deployed 7 d after the start of the experiment and remained in the field for 7 d.

more starved moths were attracted to and contacted the attract-and-kill stations ($n = 6$, df = 17, $F = 57.8$, $P < 0.01$ for attraction; $n = 6$, df = 17, $F = 47.2$, $P < 0.001$ for contact) (Table 1).

Similar results were obtained for male moth responses to attract-and-kill stations. Both starved and fed male alfalfa looper moths exhibited plume tracking in the presence of attract-and-kill stations and not in the absence of stations (Table 1) ($n = 6$, df = 17, $F = 135.0$, $P < 0.01$ for starved males; $n = 6$, df = 17, $F = 45.0$, $P < 0.01$ for fed males). Most starved and fed males that were attracted to stations contacted those stations. The numbers of males attracted to the attract-and-kill stations were significantly greater than the numbers of fed males attracted to stations ($n = 6$, df = 17, $F = 19.3$, $P < 0.01$ for plume tracking; $n = 6$, df = 17, $F = 15.6$, $P < 0.001$ for contact) (Table 1).

The mortality rate of female moths tested to attract-and-kill stations in the wind tunnel was significantly greater with starved compared with fed moths (Table 1). The mortality rate of female moths that contacted stations was not significantly different when those moths were starved versus when they were fed sugar ($n = 6$, $F = 1.0$, $P = 0.34$). The mortality rate of all males tested was significantly greater for starved versus fed males but the mortality rate of contacting males was not significantly different for starved versus fed males ($n = 6$, $F = 4.9$, $P > 0.05$). The mortality rate of male moths that contacted the stations was not significantly different when comparing male moths that were starved and male moths that had been provided sugar.

2003 Field Test. Daily captures of female alfalfa looper moths in floral lure traps after the eighth day of the experiment were nearly always numerically lower in treated plots compared with untreated plots, whereas numbers of moths in these traps were not statistically different in treated and control plots before treatment (Fig. 2). The numbers of female moths captured per day in floral lure-baited traps after deployment of attract-and-kill stations were significantly lower in treated plots compared with untreated plots ($n = 4$, df = 7, $F = 6.2$, $P = 0.05$) (Table 2). Also, fewer females were captured in floral lure traps in treated plots after station deployment (postdeployment) compared with before deployment of stations (predeployment period) in those same plots ($n = 4$, df = 7, $F = 9.8$, $P = 0.02$) (Table 2). There was no difference between numbers of females trapped in treated versus nontreated plots during the predeployment period ($n = 4$, df = 7, $F = 3.3$, $P < 0.12$) (Table 2). Finally, there was no difference between numbers of females trapped in the untreated plots during the predeployment versus post deployment periods ($n = 4$, df = 7, $F = 0.29$, $P = 0.61$) (Table 2).

There was no significant difference between the numbers of males captured in floral lure traps in untreated versus treated plots either before attract-and-kill stations were deployed ($n = 4$, df = 7, $F = 0.58$, $P = 0.48$) (Table 2) or after stations were deployed ($n = 4$, df = 7, $F = 2.3$, $P = 0.18$) (Table 2). The numbers of males captured in floral lure traps were significantly lower after the deployment of stations compared with the predeployment period (Table 2), in treated plots ($n = 4$, df = 7, $F = 6.0$, $P = 0.05$) and in control plots ($n = 4$, df = 7, $F = 0.21$, $P = 0.66$).

There was no significant difference between the numbers of male alfalfa looper moths in pheromone traps in untreated plots versus treated plots for either the pre- or the postdeployment periods ($n = 4$, df = 7, $F = 0.01$, $P = 0.92$ for predeployment; $n = 4$, df = 7, $F = 0.01$, $P = 0.97$ for postdeployment) (Table 2). There was a significant increase in the numbers of

Table 2. Mean ± SE numbers of alfalfa looper moths captured per day in alfalfa fields during the 2003 field test

	0 stations/ha	125 stations/ha
Floral lure		
Female predeployment	0.89 ± 0.21ar	1.50 ± 0.22ar
Female postdeployment	0.86 ± 0.30ar	0.11 ± 0.07bs
Male predeployment	2.32 ± 0.43ar	2.86 ± 0.59ar
Male postdeployment	0.96 ± 0.33as	0.50 ± 0.18as
Sex pheromone		
Male predeployment	4.45 ± 0.57ar	4.66 ± 0.51ar
Male postdeployment	7.38 ± 1.19as	7.20 ± 0.55as

Monitoring traps were baited with a floral lure or the sex pheromone. Attract-and-kill stations remained in plots for 7 d. Pairs of means within a row followed by the same letter (a, b) and means within a column followed by the same letter (r, s) are not significantly different ($n = 4$, $P = 0.05$; Dunnett's test).

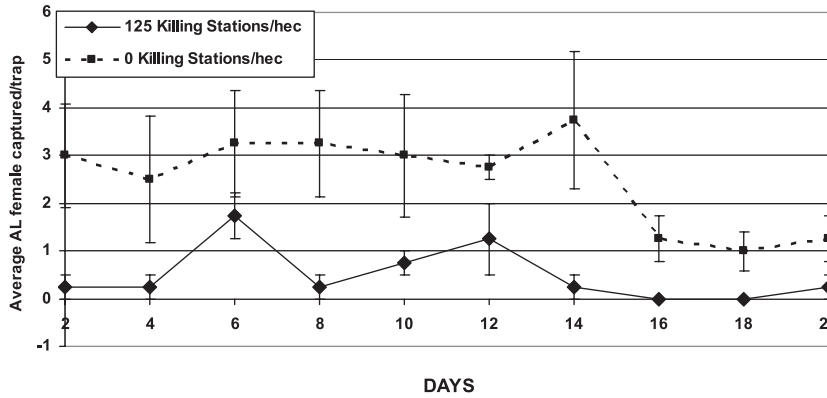

Fig. 3. Mean ± SE ($n = 4$) female alfalfa looper moths captured with feeding attractant baited traps during the 2004 growing season. Attract-and-kill stations were deployed at the beginning of the experiment and remained in the field for 20 d.

male alfalfa loopers in sex pheromone traps after station deployment in both untreated and treated plots ($n = 4$, df = 7, $F = 0.45$, $P = 0.53$ for nontreated plots; $F = 0.84$, $P = 0.39$ for treated plots) (Table 2).

2004 Field Test. The numbers of female moths captured in floral lure traps were higher throughout the 20-d duration of the experiment in treated versus untreated plots (Fig. 3). The numbers of female moths captured in floral lure-baited traps in treated plots (125 attract-and-kill stations per ha) were significantly lower than in untreated plots ($n = 4$, df = 39, $F = 41.6$, $P < 0.01$) (Table 3). Although a numerical reduction was observed, there was not a significant difference between numbers of males trapped in treated plots compared with nontreated plots ($n = 4$, df = 39, $F = 3.7$, $P = 0.06$) (Table 3).

The numbers of males captured in sex pheromone traps in treated versus nontreated plots were numerically lower, but this was not statistically significant ($n = 4$, df = 39, $F = 1.9$, $P = 0.17$) (Table 3).

Screenhouse Evaluation. Starved moths released into screenhouses with the attract-and-kill stations produced significantly fewer eggs and larvae on lettuce plants (4.3 ± 3.4) compared with starved moths in control screenhouses (287.7 ± 94.0) and moths fed a sugar solution before release in screenhouses that contained stations (238.3 ± 50.1) ($n = 3$, df = 8, $F = 6.1$, $P = 0.04$). Starved moths in the control screenhouses produced numbers of offspring on lettuce plants that were comparable with fed moths in treated screenhouses ($F = 0.4$, $P = 0.46$).

Table 3. Mean ± SE numbers of alfalfa looper moths captured per day in alfalfa hay fields during the 2004 field experiment

	0 stations/ha	125 stations/ha
Feeding attractant		
Females	1.3 ± 0.1a	0.3 ± 0.1b
Males	0.7 ± 0.1a	0.4 ± 0.1a
Sex pheromone		
Males	31.5 ± 2.5a	27.1 ± 2.0a

Monitoring traps were baited with a floral lure or the sex pheromone. Attract-and-kill stations remained in plots for 20 d. Means within a row followed by the same letter (a, b) are not significantly different ($n = 4$, $P = 0.05$; Dunnett's test).

Discussion

Results of flight tunnel experiments demonstrated efficacy of the attract-and-kill system both for attracting alfalfa looper moths and also for effecting mortality among attracted moths. The chemical attractant used, phenylacetaldehyde with β-myrcene, was previously demonstrated to be effective as a lure for trapping female and male alfalfa looper moths (Landolt et al. 2006). Our results here, using a controlled release device that emits ≈43 μg of attractant per hour (Landolt et al. 2001), provided an overall attraction rate of ≈80% of moths tested, with most moths contacting the shuttlecock of the attract-and-kill system. This response rate is comparable to those obtained in other flight tunnel evaluations of moth responses to floral attractants. Haynes et al. (1991) obtained an 85% attraction response rate and 55% source contact from male cabbage looper moths, *Trichoplusia ni* (Hübner), tested to a blend of compounds isolated from *Abelia grandiflora* Andre flowers. Heath et al. (1992a) similarly reported an attraction rate of 69 and 69% source contact for female cabbage looper moths tested to a blend of compounds from flowers of night-blooming jessamine, *Cestrum nocturnum* L. Dötterl (2004) showed a 90% attraction response rate by *Hadena bicruris* Hufnagel to lilac aldehyde isomers from *Silene latifolia* Poiret flowers. Plepys (2001) reported >80% attraction and 65% source contact by the silver Y moth, *Autographa gamma* L., in response to the flower odorant phenylacetaldehyde. The mortality rate for alfalfa looper moths contacting our attract-and-kill system in the flight tunnel was ≈90% for both males and females. Again, this result compares favorably to results obtained previously with the cabbage looper moth. Female cabbage looper moths in a field cage with a combined phenylacetaldehyde lure, methomyl pesticide formulation, and sucrose solution showed a mortality rate of 61% (Landolt et al. 1991).

Results of our flight tunnel experiments indicated that starved male and female alfalfa looper moths are significantly more attracted to the floral lure than fed moths. This finding is not unexpected, because the lure (phenylacetaldehyde and β-myrcene) is based on the odor chemistry of flowers at which these moths are thought to obtain nectar (Landolt and Smithhisler 2003). The reduced response of fed moths to the floral lure compared with starved moths supports the hypothesis that alfalfa looper moths are attracted to these compounds in search of food. Recently fed moths probably do not respond well to the floral lure because they are not hungry. Despite multiple studies of moth attraction to flowers and flower chemicals, we are not aware of other demonstrations of differences in moth responses to flower odorants in relation to prior feeding history. This effect is of concern in developing pest management applications with feeding attractants because of the possibility that moths in the field may not respond to floral lures or other feeding attractants in an attract-and-kill system when adequate sources of nectar or other foods are available.

The deployment of attract-and-kill stations in field plots resulted in reductions in the numbers of female alfalfa loopers captured in floral lure traps. We interpret this as evidence that moths attracted to the floral lures of the attract-and-kill stations were killed by contact with the pesticide formulation on the station, resulting in a lowering of the numbers of moths present and able to respond to the floral lures in the monitoring traps of those plots. A comparable approach was used by Charmillot et al. (2000), who used pheromone traps to monitor codling moths in plots treated with an attract-and-kill formulation by using sex pheromone. These reductions in numbers of moths trapped also might be interpreted to be a result of some type of disruption; that moths did not arrive at lures of the two monitoring traps because of the confusing presence of the 250 floral lures of the attract-and-kill stations of the plot.

Results for male captures in monitoring traps were very different from those for females. Although there were reductions in numbers of males captured in floral lure traps when attract-and-kill stations were deployed, compared with predeployment numbers, in the 2003 field experiment, there were also fewer males in floral lure traps in control plots. The numbers of males captured in pheromone traps were not affected significantly by the presence of attract-and-kill stations and they were similar for treated and untreated plots. These results may be due to greater movement of males than females and to a weaker male response to the floral lure, as observed in wind tunnel experiments. It is also likely that males responding to sex pheromone lures in monitoring traps came from a large area relative to our 2-ha plots. Although reductions of numbers of males captured in sex pheromone and floral lure traps would be encouraging, the primary goal of this work was to reduce the numbers of female moths in plots, thereby lowering the numbers of eggs laid.

Our results indicated both an immediate effect on female alfalfa looper moths in the field and a longevity of the effectiveness of the attract-and-kill stations. In the 2003 experiment, the knockdown effect on female moths in treated plots was clearly evident on day 8 of the test, one day after deployment of the stations. The stations also performed well over the 20-d duration of the 2004 experiment, with numbers of females trapped in treated plots nearly always fewer than in control plots throughout the tests. To be effective for that length of time, the release rate of the floral lure must be maintained and the efficacy of the permethrin formulation on the station must persist.

The experiment conducted in a screenhouse demonstrated that the attract-and-kill-stations can be effective in reducing reproduction. We interpret the reductions in alfalfa looper offspring on lettuce plants in treated assays as evidence that female moth mortality at stations prevented female oviposition on lettuce plants in the screenhouse. There is the added possibility that mortality of males at stations reduced female mating success, because males and females were unmated when released into the screenhouse, which could then have contributed to reduced reproduction in treated assays. However, there is no documentation of this relationship in our study.

Differences obtained in screenhouse tests with fed versus starved moths is consistent with the differences observed in fed versus starved moth responses in the flight tunnel to attract-and-kill stations. Starved females gave the best results in both the flight tunnel and the screenhouse, with strong responses to the floral lure and greatly reduced reproduction in the screenhouse with attract-and-kill stations. Fed females in the flight tunnel were less attracted to the floral lure, and oviposition by fed females in the screenhouse with attract-and-kill stations was similar to females in the screenhouse without attract-and-kill stations. This suggests that fed females were less responsive to the floral lure, making the stations in the screenhouse ineffective. These results support the hypothesis that starved females were indeed killed at the stations in the field and in the screenhouse, before they were able to oviposit. These findings also demonstrate a potential drawback of the use of feeding attractants such as floral lures for the attract-and-kill stations. It is likely that under some circumstances moths may have adequate food resources and may not be strongly responsive to feeding attractants. This could occur when a crop such as alfalfa blooms, or when weed species are in flower, or when other on- or off-site food sources are available. Despite this concern, results of field experiments suggest a reduction of numbers of wild female alfalfa looper moths in plots with attract-and-kill stations.

Recently, developed attract-and-kill or lure-and-kill technology has shown efficacy in the control of several lepidopteran pests, including the light brown apple moth, *Epiphyas postvittana* (Walker) (Suckling and Brockerhoff 1999), and the codling moth (Charmillot et al. 2000, Krupke et al. 2002). These studies used sex pheromones as a lure, causing mortality of males and thereby reducing the sex ratio of the population. This concept was developed as an attempt to control re-

production by reducing female mating success. The primary advantage emphasized in our studies is the attraction of both sexes when using feeding attractants. Landolt et al. (1991) developed an attract-and-kill system against the cabbage looper that provided attraction and mortality rates in laboratory and field cage assays similar to our those of our experiments. Field experiments to reduce populations of Lacanobia fruitworm, *Lacanobia subjuncta* (Grote & Robinson) in apple (*Malus* spp.) orchards used attract-and-kill stations baited with acetic acid with 3-methyl-1-butanol (Landolt 2002). At a density of 125 stations per ha, deployment of these attract-and-kill stations significantly reduced the numbers of female *L. subjuncta* moths captured in monitoring traps. Work also has been done to develop attract-and-kill methods that include feeding attractants for other types of insects, such as *Diabrotica* beetles (Metcalf et al. 1987).

Attract-and-kill stations based on feeding attractants may have drawbacks compared with a sex pheromone-based system. Sex pheromones are often species-specific lures. Feeding attractants, however, may attract an array of insects including other pests but also beneficial insects. A variety of Lepidoptera and Hymenoptera were commonly captured in our floral lure traps. Bumble bees (*Bombus* spp.); honey bees, *Apis mellifera* L.; and sweat bees (Halictidae), among others, were commonly captured in monitoring traps, and they are potentially killed at attract-and-kill stations, during the field experiments. Another drawback is that feeding attractants are not demonstrated for all moth pests, but sex pheromones are identified for most pest moth species (Mayer and McLaughlin 1991). Also, competition from other odorants present in the environment may detract from the attractiveness of feeding attractants used in attract-and-kill systems. Flowering fields might reduce the power of the lures in such attract-and-kill stations, reducing their effectiveness. During the 2004 experiment, alfalfa plants began flowering in one of the test replicates. Perhaps this is why we observed reductions in the number of moths trapped in both treated and control plots during the 2004 field season between day 14 and day 16 (Fig. 3).

The density of attract-and-kill stations used in our experiments, 125 stations per ha, is low compared with some other studies of attract-and-kill technologies. Charmillot et al. (2000) used 1,000 droplets per ha to control codling moth with sex pheromone, and Losel et al. (2000) used 7,500 droplets per ha, also to control codling moth with sex pheromone. The density of stations required to be effective may be related in part to the type of lure used and to the sex of the moth targeted. The killing of males as a control strategy requires the killing of a large percentage of males before significant impact on female mating might be attained (Knipling 1979). If females are killed when reproductively active or prereproductive, then the removal of those females from the population might directly reduce oviposition Thus, it is expected that a higher density of attract-and-kill stations are required for male lure-based systems versus systems based on lures that attract both sexes or attract females.

Even though our results are encouraging, additional studies are needed to directly assess effects of attract-and-kill stations on larvae in field plots. Commercially grown alfalfa hay fields might not be the most suitable sites for such studies because of the fast cutting cycle during the summer when moths are flying, which may keep alfalfa looper reproduction depressed. The alfalfa looper is also a serious pest of vegetable crops such as crucifers and lettuce (Brewer 1995), which may provide more reliable and measurable larval infestations for such experiments.

This attract-and-kill approach with a lure targeting females may also be feasible for a number of other pestiferous noctuid moth species. Floral based attractants are known for cabbage looper; soybean looper *Pseudoplusia includens* (Walker); silver Y moth; corn earworm, *Helicoverpa zea* (Boddie); cotton bollworm, *Helicoverpa armigera* (Hübner); and tobacco budworm, *Heliothis virescens* (F.) (Cantelo and Jacobson 1979, Haynes et al. 1991, Heath et al. 1992a, Pair and Horvat 1997, Lopez et al. 2000, Plepys 2001). The feeding attractant acetic acid with 3-methyl-1-butanol is effective in luring spotted cutworm, *Xestia c-nigrum* (L.); *L. subjuncta;* bertha armyworm, *Mamestra configurata* (Walker); and true armyworm, *Pseudaletia unipuncta* (Haworth) (Landolt 2000, Landolt and Higbee 2002). Other types of chemical lures for female moths include the male pheromone of the cabbage looper (Heath et al. 1992b) and a host kairomone for the codling moth (Light et al. 2001). Perhaps these feeding attractants, pheromones, and host kairomones that attract female moths will provide opportunities to investigate and develop other attract-and-kill systems. However, the strength of the attractants (the power to attract) for these moths also would affect the efficacy of any attract-and-kill technology.

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